ZOOLOGICAL SCIENCE 5: 385–395 (1988)

Glandular Epithelium Induced from Urinary Bladder Epithelium of the Adult Rat Does Not Show Full Prostatic Cytodifferentiation

NAOYA SUEMATSU, HIROYUKI TAKEDA and TAKEO MIZUNO

Zoological Institute, Faculty of Science, University of Tokyo, Hongo, Tokyo 113, Japan

ABSTRACT—Urinary bladder epithelium of the adult rat formed prostate-like glands, when combined with fetal urogenital sinus mesenchyme in culture and grafted beneath the renal capsule of male rat hosts. With the progression of the gland formation, the bladder epithelium lost its alkaline phosphatase activity and antigenicity against anti-functional bladder epithelium-antiserum. Like the normal prostate, the induced epithelium expressed acid phosphatase, nonspecific esterase and antigenicity against anti-human prostatic acid phosphatase-antiserum, but hardly expressed androgen receptors nor an antigen against anti-4-week prostate epithelium-antiserum. The SDS-PAGE patterns of total proteins in the induced glandular epithelium and in the normal epithelia of the urinary bladder and prostate revealed that the induced glandular epithelium loses some bands identified in the bladder epithelium and comes to express other bands similar to but not identical with those of the normal prostatic epithelium, suggesting that the induced gland does not differentiate as completely as the normal prostate, and this result is linked with extremely reduced androgen receptors in the induced epithelium.

INTRODUCTION

The prostate glands are one of the mammalian male accessory sex organs and develop from their rudiments in the urogenital sinus, which are recognized as epithelial buds projecting into the surrounding mesenchyme. Lasnitzki and Mizuno [1] have shown that the sinus epithelium requires both androgens and the sinus mesenchyme to form prostatic buds *in vitro*. The androgen receptors observed by steroid autoradiography [2] concentrate mainly in the sinus mesenchyme but are absent in the sinus epithelium until the lumen starts to be formed. It is likely, therefore, that the androgens stimulate first the sinus mesenchyme, and that the activated mesenchyme produces substances, which stimulate the epithelium to form buds, though their nature is yet unknown.

The urinary bladder epithelium could form prostatic glands when combined with the sinus mesenchyme [3]. Furthermore, the bladder epithelium not only formed morphologically recognizable prostatic glands but also differentiated biochemically into prostatic glandular epithelium as assessed by protein profiles in two-dimentional gel electrophoresis [4].

In this paper we examined the nature of the prostate-like glands induced in the adult bladder epithelium and whether the induced glands accomplish full prostatic differentiation. The degree of cytodifferentiation was judged by the types and amount of proteins counted in slab gel electrophoresis and by immunochemistry of the tissue. Further an attempt was made to relate the type of cytodifferentiation to the presence or absence of androgen receptors.

Accepted October 22, 1987
Received August 28, 1987

MATERIALS AND METHODS

Animals and tissues

Inbred rats (Fischer 344, Charles River Japan Inc., Kanagawa) were mated during the night and copulation was confirmed by the presence of

spermatozoa in the vaginal smears on the following morning. Noon of the day was recorded as 0.5 days of pregnancy.

Urinary bladder and uterus were obtained from adult pregnant rats. Urogenital sinuses, rectums and stomachs were excised from 16.5-day male and female fetuses. Rudiments of the seminal vesicle were dissected out from 19.5-day male fetuses. The excised urogenital sinus was then separated into ventral and dorsal halves that develop ventral and dorsal lobes of the prostate respectively.

Collection of epithelium and mesenchyme

The adult bladder and uterus were treated with collagenase (Worthington Biochemical Corp., Code CLS) 0.03% in Tyrode's solution for 2 hr at 37°C and the fetal urogenital sinus, rectum, stomach and rudiments of seminal vesicle were treated with 0.06% collagenase for 30 min at 37°C. The collagenase-treated tissues were washed in 50% fetal bovine serum (FBS) in Tyrode's solution for 2 hr with three changes, and the epithelium was separated from the mesenchyme with two watchmaker's forceps under a stereomicroscope (×20).

Culture method

A small fragment of the epithelium was placed upon a piece of the mesenchyme put on a membrane filter (Millipore Corp., pore size 0.8 μm), which was put on a stainless grid placed in a glass dish filled with medium 199 with Earle's salts (GIBCO) containing 20% FBS to the level of the membrane filter, cultured in a CO_2 incubator (5% CO_2, 95% air, at 37°C) overnight and then the recombinate was grafted beneath the kidney capsule of syngeneic adult male hosts (8–20 weeks old) (designated as "*in vivo* cultivation"). After 3–5 weeks, the grafts were harvested for examination.

Enzyme histochemistry

Alkaline phosphatase The grafts were fixed in ice-cold 80% ethanol for 2 hr and embedded in paraffin (m.p. 46–48°C). The alkaline phosphatase was detected by the tetrazolium reaction [5] at pH 9.2–9.4.

Acid phosphatase and nonspecific esterase The grafts were fixed in ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2–7.4) for 2 hr, washed overnight in several changes of 5% sucrose at 4°C and frozen in isopentane (−190°C) chilled with liquid nitrogen. The acid phosphatase was assessed by azo-coupling method [6] at pH 5, and the nonspecific esterase, by the azo-coupling method [7] at pH 5.

Immunohistochemistry

As the first ligands for the indirect immunofluorescence methods, the following antibodies were used: a rabbit polyclonal anti-human prostatic acid phosphatase-antibody (Miles-Yeda Ltd.), which stained patches of epithelial cells more intensely in the ventral prostate than in the dorsal one; mouse polyclonal anti-rat ventral or dorsal prostatic epithelium-antibodies of which antigen was homogenate of glandular cells of 4-week rat ventral or dorsal prostate respectively and the antibody was prepared in our laboratory according to the method of McKeehan *et al.* [8]; and a mouse polyclonal anti-rat urinary bladder epithelium-antibody prepared in our laboratory. Each antibody was applied to the sections of various tissues including urinary bladder, ventral and dorsal prostate, seminal vesicle, liver and kidney and thus confirmed to be specific to the tissues concerned.

Tissues were fixed in methanol (−20°C) for 30 min, replaced in ethanol (−20°C) for 30 min, embedded in Polyester wax (melting point 37°C, BDH Chemicals Ltd.), and sectioned at 6 μm. They were treated with the primary antibody for 1 hr at room temperature and exposed to second antibodies: Goat anti-rabbit IgG antibody (Miles) or goat anti-mouse IgG antibody (Cappel), both conjugated with fluorescein isothiocyanate. The sections were examined with an Olympus epifluorescence microscope (BH2-RFK). After observation, the sections were washed in phosphate-buffered saline and stained with haematoxylin-eosin (HX-E).

Steroid autoradiography

Tissues and grafts were labelled with [1, 2, 4, 5, 6, 7-^{3}H] dihydrotestosterone and then subjected to the thaw-mount autoradiographic technique. The details of steroid autoradiographic techniques

have been described previously [2].

Polyacrylamide gel electrophoresis (PAGE) and immunoblotting

Tissues were treated with collagenase in Ca^{2+}, Mg^{2+}-free Tyrode's solution for 1.5 hr at 37°C, washed with gentle stirring in Ca^{2+}, Mg^{2+}-free Tyrode's solution for 30 min at room temperature. Then isolated glandular epithelial cells were pulverized in a stainless steel mill chilled with liquid nitrogen, dissolved in a SDS sample buffer including 5% (v/v) 2-mercaptoethanol and 2.3% (w/v) sodium dodecyl sulphate (SDS), and subjected to electrophoresis on a polyacrylamide 4/20 gradient gel with SDS (Daiichi Pure Chemicals Co.) according to Laemmli [9]. As molecular-weight markers, LMW calibration kit (Pharmacia) was used. After electrophoresis, proteins on the gels were either stained with silver or transferred electrophoretically to Durapore filters (Millipore) according to Towbin *et al.* [10]. The filters were then treated first with the antibody to be examined, and next with goat anti-mouse IgG antibody conjugated with horseradish peroxidase (Miles), washed, and treated with 4-chloro-1-naphthol in the presence of hydrogen peroxide.

RESULTS

Morphological differentiation There was no contamination with epithelial cells in the isolated urogenital sinus mesenchyme throughout the experiments examined in serial sections of the separated components (14 cases in total). All of the 17 homotypic recombinates composed of fetal ventral sinus epithelium and its mesenchyme developed fully differentiated ventral prostate glands with high secretory activity, when grown for 3.5 weeks beneath the kidney capsule of normal male hosts (Fig. 1), but all 5 recombinates failed to develop prostatic glands when grown in castrated male rats. Adult bladder epithelium recombined with fetal ventral sinus mesenchyme underwent morphological changes after *in vivo* cultivation in normal male hosts: after 1 week of cultivation, epithelial buds projected into the surrounding mesenchyme in 5 recombinates out of 5 (Figs. 2a and 2a′); after 2 weeks, lumina were formed in the extended buds (Fig. 2b); after 3 weeks, prostate-like glands were formed, but they were lined with epithelium generally shorter than that found in the homotypic recombinates and were rich in stroma and poor in secretion (Fig. 2c) in all 37 recombinates. After 8 weeks, there was no further differentiation in all 8 recombinates (Fig, 2d). In the castrated male hosts, the bladder epithelium was maintained in the same state as at the onset of culture and no glands were formed in all 12 recombinates even in the presence of the sinus mesenchyme.

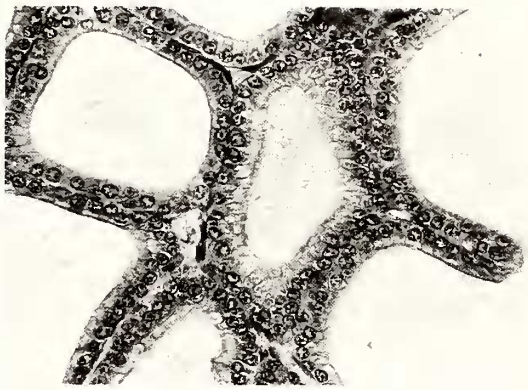


FIG. 1. A homotypic recombinate of fetal ventral sinus epithelium and its mesenchyme grown for 3.5 weeks in an adult male host. ×280.

Mesenchymes of 16.5-day ventral and dorsal sinuses and of 19.5-day seminal vesicle induced prostate-like glands from the adult bladder epithelium, though the glands were morphologically different to some extent according to the kind of the recombined mesenchymes (Fig. 3a, b, and c), but fetal rectal mesenchyme did not. The other examined epithelia combined with fetal ventral sinus mesenchyme did not form any glandular structure even after 4 weeks' *in vivo* cultivation: In all 3 recombinates, adult uterine epithelium made only a tube; Fetal rectal epithelium formed a vesicle, with epithelium that developed many goblet cells in all 4 recombinates; Fetal stomach epithelium formed a vesicle, lined with mucous epithelium characteristic of the adult stomach epithelium in all 3 recombinates.

Functional differentiation The enzyme activities were seen to change during the prostate-like gland formation. The epithelium of the adult

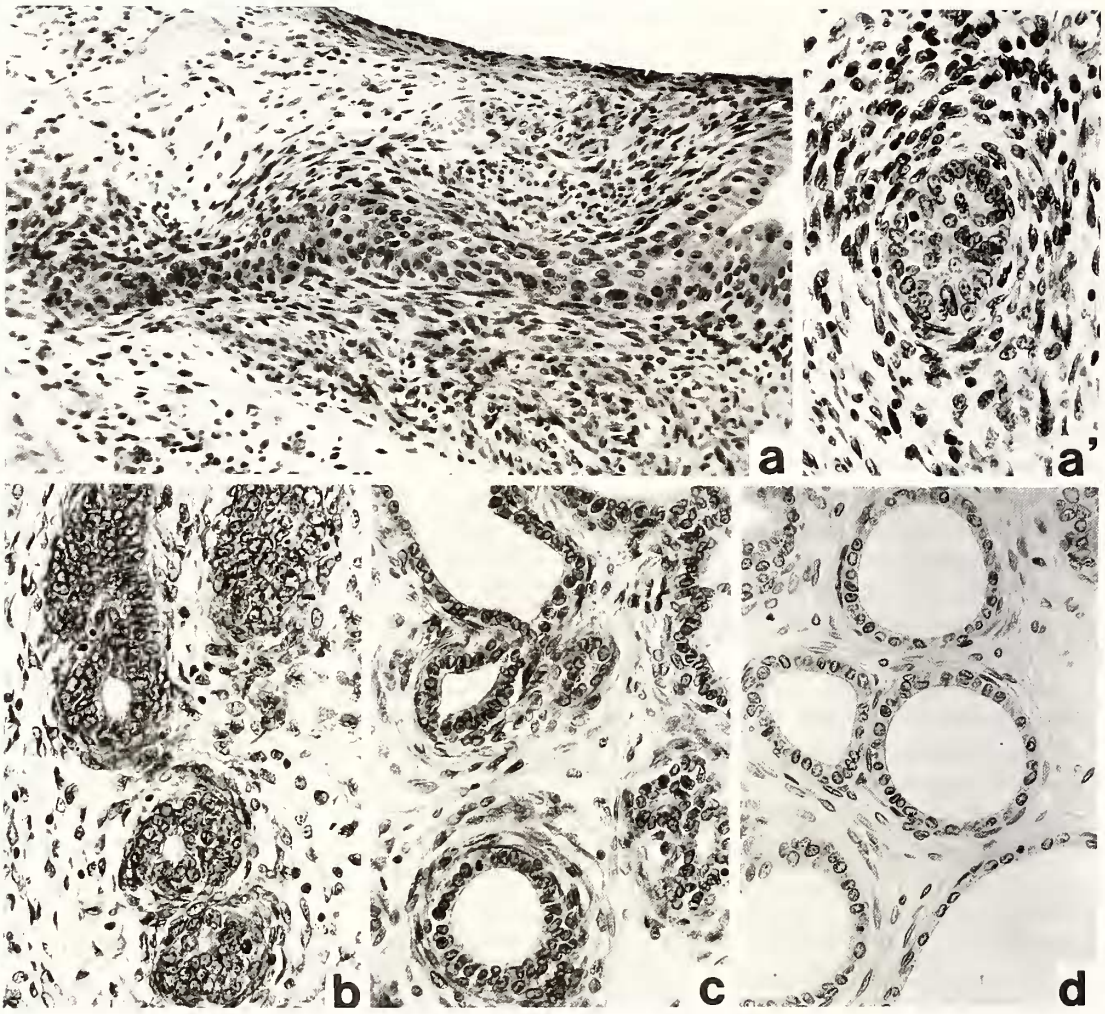


FIG. 2. Recombinates of adult bladder epithelium and fetal ventral sinus mesenchyme. (a) 1 week, ×215; (b) 2 weeks, ×270; (c) 3.5 weeks, ×270; (d) 8 weeks, ×135, cultured in adult male hosts. (a') A cross section of the epithelial bud as seen in (a), ×340.

bladder was positive for alkaline phosphatase activity (Fig. 4a) and negative for both acid phosphatase (Fig. 4b) and nonspecific esterase (Fig. 4c) activities. In contrast, the glandular epithelium induced in the bladder epithelium by the fetal ventral sinus mesenchyme lost its alkaline phosphatase activity (Fig. 4d) but showed acid phosphatase (Fig. 4e) and nonspecific esterase (Fig. 4f) activities just like the acinar epithelium of the normal ventral prostate, which was negative for alkaline phosphatase (Fig. 4g) and positive for both acid phosphatase (Fig. 4h) and nonspecific esterase (Fig. 4i). The stromal cells immediately adjacent to the acinar epithelium were positive for alkaline phosphatase both in the induced and normal prostatic glands. The glandular structure induced by the mesenchymes of fetal dorsal sinus and seminal vesicle showed similar histochemical features as those induced by the mesenchyme of the fetal ventral sinus. There was thus no difference between the heterotypic and homotypic recombinates as far as histochemical activities of these enzymes were concerned.

The immunohistochemical study also suggested

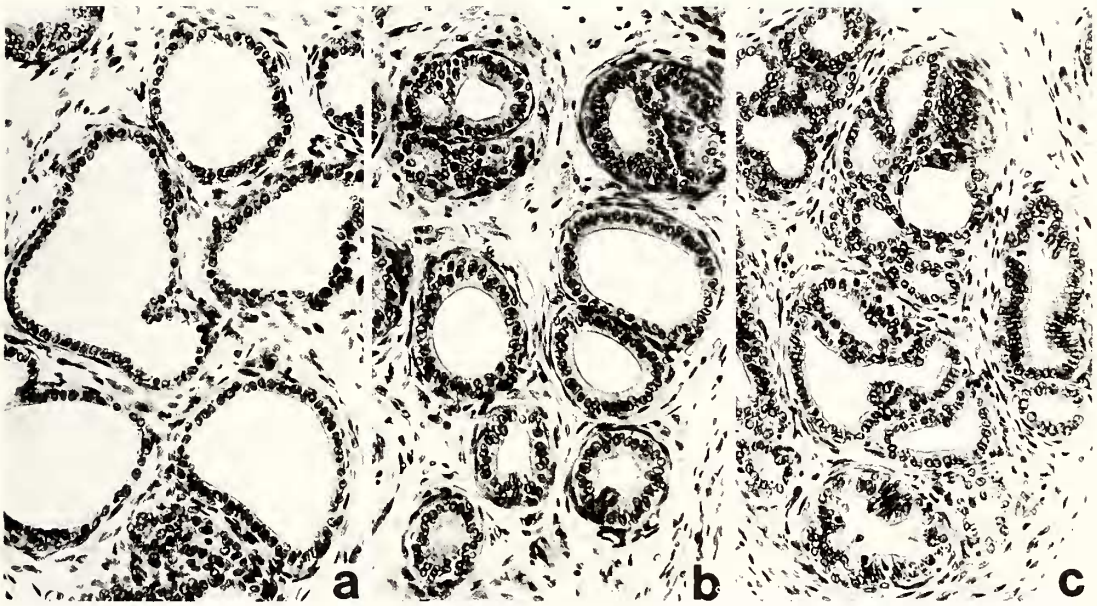


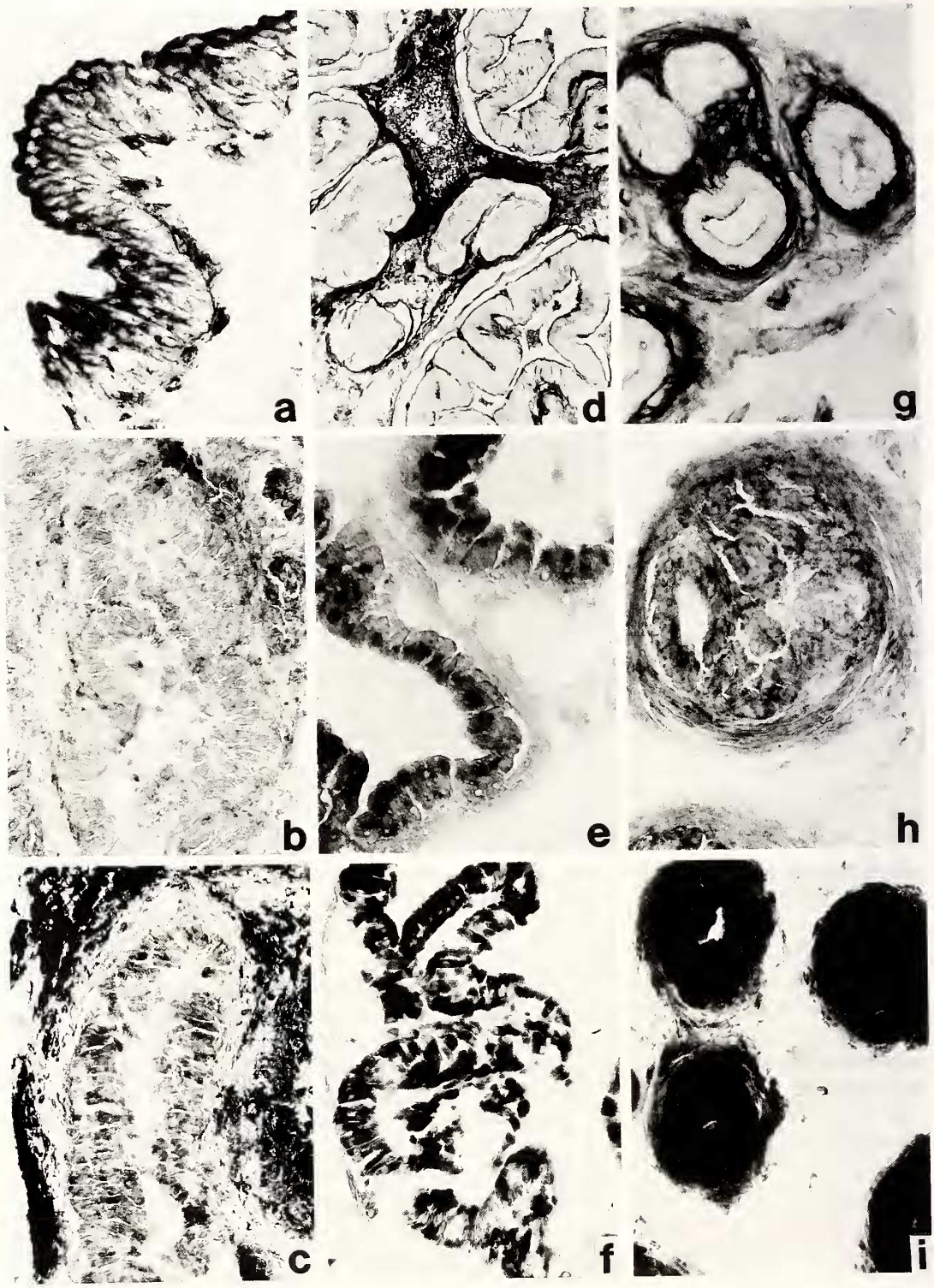
FIG. 3. Heterotypic recombinates of adult bladder epithelium and 16.5-day ventral sinus mesenchyme (a), dorsal sinus mesenchyme (b), or 19.5-day seminal vesicle mesenchyme (c) cultured in adult male hosts. ×215.

that heterotypic functional differentiation occurred in the induced glands. The bladder epithelium reacted with our anti-bladder epithelium-antiserum (Fig. 5) even after *in vivo* culture of 4 weeks as far as it maintained its original form seen at the time of recombination with sinus mesenchyme. The antigenicity against the antiserum was lost in the epithelium, when induced to form glandular epithelium (Fig. 6). This result suggests that the bladder epithelium dedifferentiated to express specific enzyme activities following the morphological gland formation.

Most of the induced glandular epithelium was found to be positive against anti-human prostatic acid phosphatase-antiserum (Fig. 7), although some glandular cells as well as the normal counterparts were negative. Oddly enough it did not express antigenicity against the anti-ventral prostatic epithelium-antiserum (Fig. 8a), which intensely stained the normal ventral prostatic epithelium in the control explant (Fig. 8c) but only faintly the ventral prostate 5 days after castration (Fig. 8d). In the immunoblotting, the anti-ventral prostatic epithelium-antiserum recognized many types of proteins specific to the ventral prostatic epithelium (Fig. 9). The induced glandular epithelium did not respond to anti-dorsal prostatic epithelium-antiserum, which reacted with the normal dorsal prostatic epithelium (Fig. 8e).

The steroid autoradiography showed heavy nuclear labelling with [^{3}H] dihydrotestosterone (DHT) in homotypic explants of sinus epithelium with its mesenchyme (Fig. 10a). In contrast, the glandular epithelium induced from bladder epithelium showed no preferential nuclear uptake of the steroid, but the surrounding mesenchymal cells showed heavily labelled nuclei in all 5 recombinates (Fig. 10b). In the competition experiments in which the tissues were incubated both with the radioactive steroid and with a 400-fold excess of unlabelled steroid, the uptake of radioactive steroid by nuclei was completely abolished.

We also compared the total proteins of the isolated glandular epithelial tissues with each other, which were separated by SDS-PAGE. The induced glandular epithelium differed qualitatively and quantitatively from the bladder epithelium but not from the ventral prostatic epithelium. The induced glandular epithelium lost the bladder epithelium-specific proteins (B, B′, Fig. 11), and showed prostatic epithelial proteins (P, P′). Moreover the induced glandular epithelium ex-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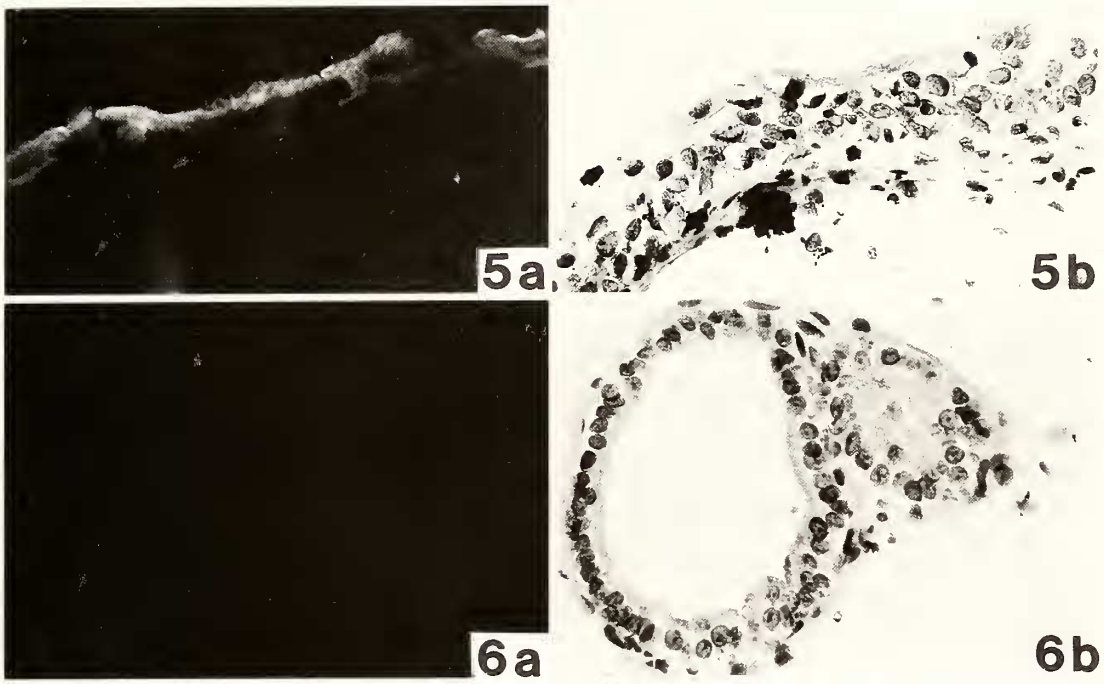

FIGS. 5. and 6. Immunofluorescence with anti-bladder epithelium-antiserum (a) and HX-E (b) preparations of normal adult bladder (Fig. 5) and induced glands in the recombinates of adult bladder epithelium and fetal sinus mesenchyme (Fig. 6). ×270.

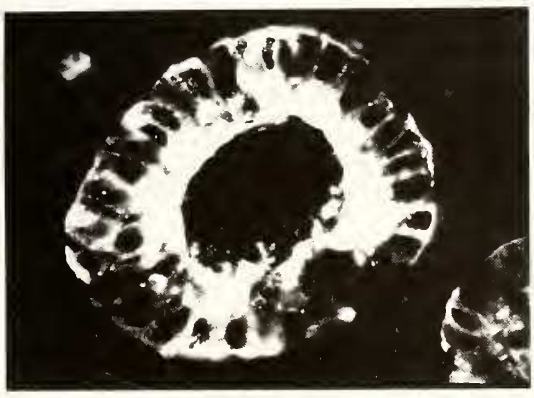

FIG. 7. Immunofluorescence with anti-human prostatic acid phosphatase-antiserum. The bladder epithelium-derived acinar epithelium showed intense reaction in the apical cytoplasm. ×480.

pressed the ventral prostatic epithelial protein (V) but not the dorsal prostatic epithelial proteins (D, D′), even when the bladder epithelium was recombined with dorsal sinus mesenchyme.

DISCUSSION

In the present study, we found that in recombinates of fully differentiated adult rat bladder epithelium with sinus mesenchyme, cultured in the presence of androgens, the bladder epithelium formed prostate-like glandular epithelium. The stratified transitional bladder epithelium which is normally alkaline phosphatase positive changed to a simple columnar glandular epithelium, became alkaline phosphatase negative and acquired acid

FIG. 4. Histochemical assay of alkaline phosphatase (a, ×280; d, ×140; g, ×270), acid phosphatase (b, ×140; e, ×340; h, ×270) and nonspecific esterase (c, ×140; f, ×250; i, ×380). Dark areas indicate the enzyme positive sites. The features of these histochemical markers expressed in the glandular epithelium induced in the recombinates composed of adult bladder epithelium and fetal ventral sinus mesenchyme (d, e, f) were similar to those of acinar epithelium of normal ventral prostate (g, h, i) but different from those of adult bladder epithelium (a, b, c).

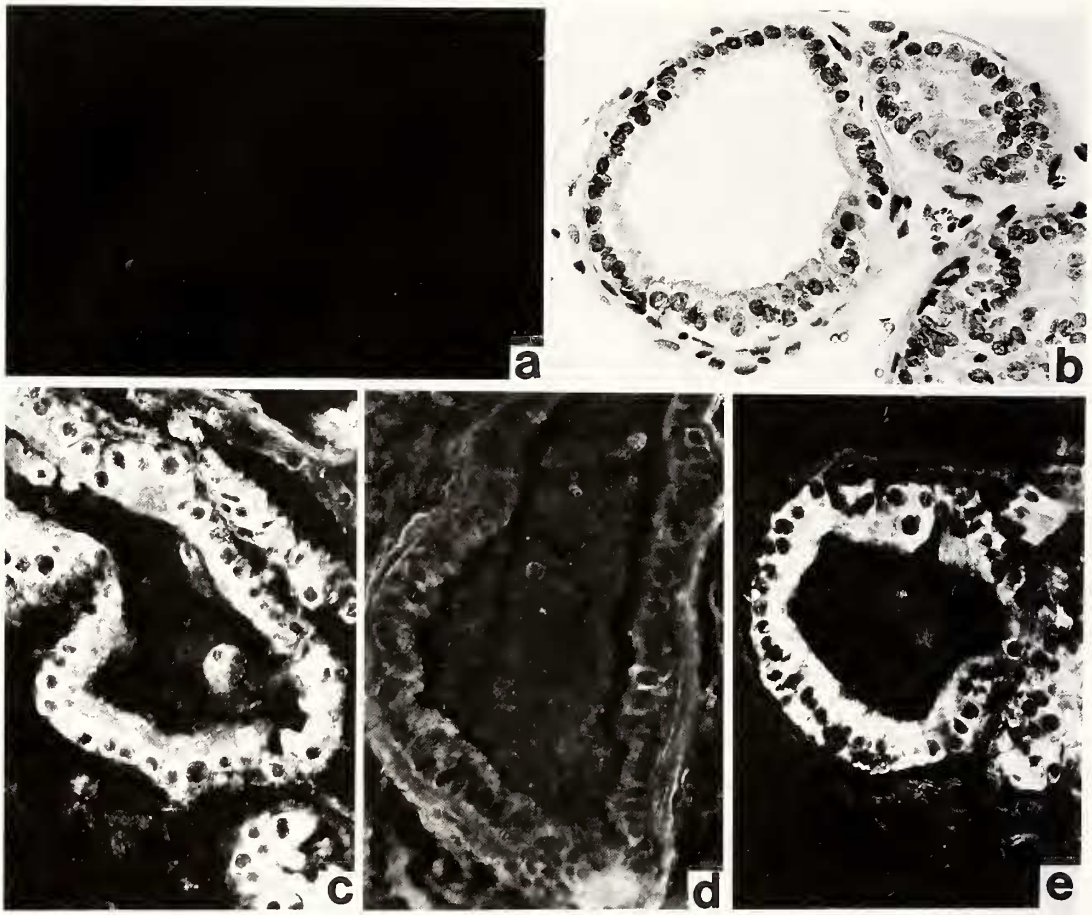

FIG. 8. Immunofluorescence with anti-ventral prostatic epithelium-antiserum of the induced glandular epithelium of bladder origin (a), ventral prostate gland in the control explant composed of sinus mesenchyme and its epithelium (c) and ventral prostate excised 5 days after castration (d). HX–E preparation (b) is the same with (a). Immunofluorescence with anti-dorsal prostatic epithelium-antiserum of normal dorsal prostate (e). ×270.

phosphatase, nonspecific esterase and prostatic acid phosphatase antigen. Heterotypic morphogenesis has been reported in the studies of epithelio-mesenchymal interactions, but sometimes it was not accompanied by heterotypic cytodifferentiation [11, 12]. However, the present study revealed that cytochemical differentiation took place concomitant with heterotypic morphogenesis, and the mesenchyme played the decisive role both in the morphogenesis and in the cytodifferentiation of the epithelium.

The ventral-, dorsal- and coagulating-lobes of prostates develop from the urogenital sinus and the seminal vesicle, from the basal region of the Wolffian ducts. Although they are similar in histological appearance, closer examination revealed differences among them. For instance, the acini of the ventral prostate are tightly packed in very small amounts of stroma and are lined with columnar epithelial cells that have basally located nuclei and a prominent supranuclear clear area that corresponds to the location of the Golgi apparatus, while the acini of the dorsal prostate are loosely distributed within large amounts of stromal tissue and are lined mainly with cuboidal epithelial cells with centrally placed nuclei and a supranuclear clear area as described by Jesik *et al.* [13].

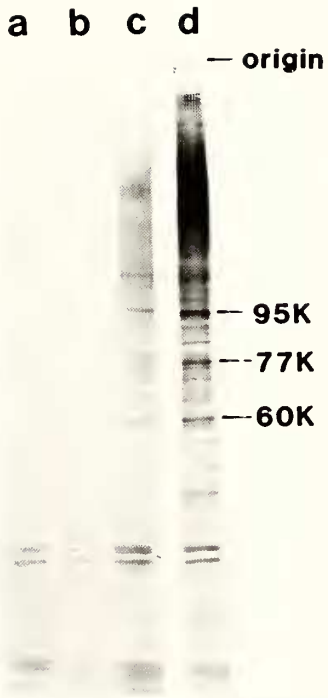

FIG. 9. Immunoblotting with anti-ventral prostatic epithelium-antiserum after electrophoresis on the gel containing SDS of crude extract of adult bladder epithelium (a), glandular epithelium in the recombinates composed of adult bladder epithelium and fetal ventral sinus mesenchyme (b), glandular epithelium in the control explants of fetal ventral sinus epithelium and its mesenchyme (c) and normal ventral prostatic epithelium (d). Ten μg of protein were applied to each lane.

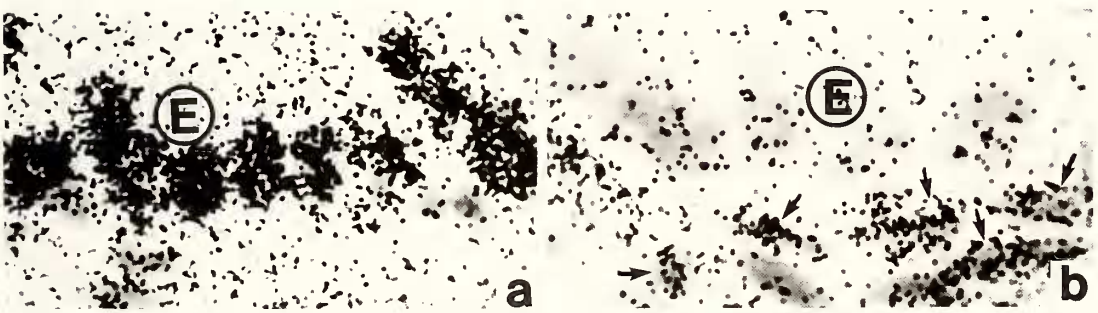

FIG. 10. Autoradiographs of explants incubated with [^{3}H]DHT. Sections were stained with HX. The exposure period was 4 weeks. E, glandular epithelium. (a) Prostate gland formed in a control explant of fetal sinus. Epithelial cells exhibited intense nuclear labelling, while the intensity of labelling in the mesenchyme surrounding the epithelium is weaker. ×1350. (b) Prostate-like gland induced in a recombinate composed of adult bladder epithelium and fetal sinus mesenchyme. Epithelial cells showed no strong nuclear concentration of grains, while the mesenchymal cells immediately beneath the epithelium (arrows) showed heavy accumulation on nuclei. ×1350.

Cunha *et al.* [3] showed that prostatic morphogenesis occurred in heterotypic recombinates of adult bladder epithelium and fetal sinus mesenchyme, but they did not mention which region of the sinus mesenchyme was used. In the present study, we used three precisely defined mesenchymal regions isolated from 16.5-day ventral and dorsal sinus and from 19.5-day rudiments of seminal vesicle and found that our results agreed mostly with those of Cunha *et al.* except in a few details: In all grafts, glands rich in stroma and poor in secretion were formed, irrespective of the region of the mesenchyme used; When the sinus mesenchyme of the ventral or dorsal region was

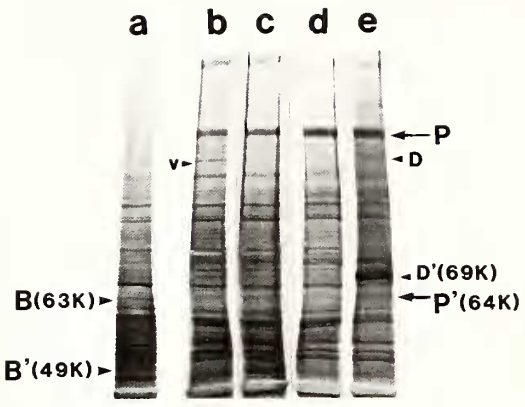

FIG. 11. SDS-electrophoretic patterns of proteins in adult bladder epithelium (a), acinar epithelium of recombinates composed of adult bladder epithelium and fetal ventral sinus mesenchyme (b) or fetal dorsal sinus mesenchyme (c), acinar epithelium of control grafts of ventral sinus (d) or dorsal sinus (e). Ten μg of protein were applied to each lane. Both (b) and (c) lost proteins B, B' and expressed P, P', V but not D, D'.

used as an inductor, the acini formed in the recombinates resembled developing prostate in the histological character: the acini were loosely arranged within the supporting stroma and lined with cuboidal epithelial cells containing a centrally located round nucleus. When the mesenchyme of the seminal vesicle was used instead of the sinus mesenchyme, the acinus epithelium possessing long nuclei seen in the seminal vesicle was also induced (Fig. 3c).

Neubauer *et al.* [4] reported that the induced acinar epithelium in the recombinates composed of adult bladder epithelium and fetal sinus mesenchyme acquired androgen receptors but that the androgen binding activities were significantly lower than those of the adult ventral prostate. We assume that the androgen binding activities they detected in the experimental grafts lies principally in the stroma rather than the epithelium. Because our autoradiographic studies have shown that androgens scarcely bind to the induced glandular epithelium in the heterotypic recombinates but mainly to the stromal cells, particularly, those in the immediate vicinity of the epithelium (Fig. 10b). Similar results have been obtained with the epithelia of androgen-receptor defective *Tfm* mice: the *Tfm* bladder epithelium as well as the sinus epithelium [14] formed prostate-like glands under the influence of normal sinus mesenchyme and expressed acid phosphatase and nonspecific esterase activities. It may, therefore, be assumed that the induced glandular epithelium is devoid of androgen receptors.

The function of the androgen receptors in the acinar epithelium is still uncertain. In adult ventral prostate glands excised 5 days after castration the reaction against anti-ventral prostatic epithelium-antiserum was significantly lowered (Fig. 8d), suggesting that the antibody recognized mainly the androgen-dependent proteins. Our results showed that the induced glandular epithelium possessed neither normal androgen receptors nor antigenicity against the anti-ventral prostatic epithelium-antibody. It seems, therefore, that the epithelial androgen receptors are necessary for the synthesis of androgen-dependent proteins responsible for epithelial function. When adult bladder epithelium was combined with dorsal sinus mesenchyme, the induced glandular epithelium did not possess D′ (69 K)-protein specific to dorsal prostatic epithelium (Fig. 11). Though the molecular weight of D′ is similar to serum albumin, serum contamination is never expected, because we did not use serum in the procedure of sample preparation, and D′ is specific to the lane (e). This suggests that the androgen receptors also play an important role in the synthesis of lobe specific proteins.

It can be concluded that adult bladder epithelium is able to respond to the stimulation of androgen-activated sinus mesenchyme and loses the characteristic proteins for the bladder epithelium and forms glandular structures with newly induced enzyme activities similar to those of the normal prostate. The induced epithelium, however, is unable to attain full prostatic cytodifferentiation nor express lobe specificity due to a lack of normal androgen receptors.

ACKNOWLEDGMENTS

We thank Dr. Ilse Lasnitzki of the Strangeways Research Laboratory for constructive criticism and help in the preparation of the manuscript. This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

REFERENCES

1 Lasnitzki, I. and Mizuno, T. (1977) Induction of the rat prostate gland by androgen in organ culture. J. Endocrinol., **74**: 47–55.

2 Takeda, H., Mizuno, T. and Lasnitzki, I. (1985) Autoradiographic studies of androgen-binding sites in the rat urogenital sinus and postnatal prostate. J. Endocrinol., **104**: 87–92.

3 Cunha, G. R., Fujii, H., Neubauer, B. L., Shannon, J. M., Sawyer, L. and Reese, B. A. (1983) Epithelial-mesenchymal interactions in prostatic development. 1. Morphological observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. J. Cell Biol., **96**: 1662–1670.

4 Neubauer, B. L., Chung, L. W. K., McCormick, K. A., Taguchi, O., Thompson, T. C. and Cunha, G. R. (1983) Epithelial-mesenchymal interactions in prostatic development. 2. Biochemical observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. J. Cell Biol., **96**: 1671–1676.

5 McGadey, J. (1970) Detection methods II. Hydrolases. Tetrazolium reaction. In "Enzyme Histochemistry (1979)". Ed. by Z. Lojda, R. Gossraw and T. H. Schiebler, Springer-Verlag, Berlin, pp. 61–63.

6 Lojda, Z. (1979) Detection methods II. Hydrolases. Simultaneous azo-coupling with esters of the naphthol AS series. In "Enzyme Histochemistry (1979)". Ed. by Z. Lojda, R. Gossraw and T. H. Schiebler, Springer-Verlag, Berlin, pp. 72–75.

7 Davis, B. J. and Ornstein, L. (1959) Detection methods 11. Hydrolases. Simultaneous azo-coupling with 1-naphthyl acetate. In "Enzyme Histochemistry (1979)". Ed. by Z. Lojda, R. Gossraw and T. H. Schiebler, Springer-Verlag, Berlin, pp. 109–110.

8 McKeehan, W. L., Rosser, M. P., Glass, H. A. and Fast, D. (1980) Prostatic binding protein: An androgen-dependent marker for prostate epithelial cells. Biochem. Biophys. Res. Commun., **95**: 674–681.

9 Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature, **227**: 680–685.

10 Towbin, H., Staehelin, T. and Gordon, J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA, **76**: 4350–4354.

11 Sakakura, T., Sakagami, Y. and Nishizuka, Y. (1979) Persistence of responsiveness of adult mouse mammary gland to induction by embryonic mesenchyme. Dev. Biol., **72**: 201–210.

12 Yasugi, S. (1984) Differentiation of allantoic endoderm implanted into the presumptive digestive area in avian embryos. A study with organ-specific antigens. J. Embryol. Exp. Morphol., **80**: 137–153.

13 Jesik, C. J., Holland, J. M. and Lee, C. (1982) An anatomic and histologic study of the rat prostate. The Prostate, **3**: 81–97.

14 Mizuno, T., Takeda, H. and Suematsu, N. (1986) Récepteurs d'androgènes de l'épithélium au cours de l'induction des glandes prostatiques à partir de l'épithélium du sinus urogénital de la Souris Tfm-mutant. C. R. Soc. Biol., **180**: 593–595.